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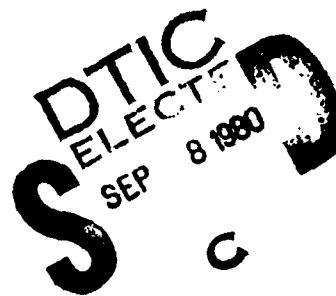# The Bacterial Flora of Incipient Occlusal Lesions in Naval Recruits

BY
J. C. MEIERS
M. R. WIRTHLIN, JR.
I. L. SHKLAIR

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The Bacterial Flora of Incipient Occlusal Lesions
in Naval Recruits

J. C. MEIERS
M. R. WIRTHLIN, JR.
I. L. SHKLAIR

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Approved and released by:

M. R. WIRTHLIN, JR.
Captain, DC, USN
Commanding Officer

Dental caries is a bacterial disease in which the prime etiological agent(s) responsible for its initiation and progression are still under investigation (1).

The bacterial colonization of smooth tooth surfaces has been well documented. Most major investigations have dealt with comparisons between the microflora found in plaque samples from carious versus non-carious teeth. The studies of Gibbons, et al. (2), Littleton, Kakehashi, and Fitzgerald (3), Loesche, et al. (4), Shklair, Keene, and Simonson (5), Hoerman, Keene, and Shklair (6), and Ikeda, Sandham, and Bradley (7) have all indicated a statistically significant increase in the numbers of acidogenic organisms (mainly Streptococcus mutans and in some studies lactobacilli) in the plaque samples from carious versus non-carious teeth. The methods employed in obtaining plaque samplings from these studies - dental floss, curettes and explorers from smooth surfaces and stainless steel wire and explorers for the occlusal surfaces - shed little light on what was occurring beneath the plaque and in the carious portion of the tooth.

Loesche and Syed (8) studying Strep. mutans and lactobacilli, Shovlin and Gillis (9) studying lactobacilli, and Batty (10) studying actinomyces, all found that the numbers and types of organisms present in carious dentin were different from the organisms located in the overlying plaque.

Pits and fissures of teeth represent the most common sites for dental caries, yet they are the areas where caries preventive measures are the least effective (11).

Epidemiological studies have consistently indicated that dental caries occurs more frequently in occlusal fissures rather than on smooth surfaces, which have up to now received the major share of study (12,13). From a public health viewpoint, the incidence of caries in fissures is affected less, than that of smooth surfaces, by various preventive measures such as water fluoridation (14) and sodium fluoride dentifrices or mouthwashes (15).

The anatomical configuration of occlusal pits and fissures allows for the physical entrapment of bacteria which do not have the ability to adhere to smooth surfaces as does Strep. mutans. The conditions in a fissure also favor the availability of substrate for long periods of time due to the inability to cleanse these areas. These unique features may allow many of the microorganisms of the mouth to be cariogenic, if not by themselves, then by complementing one another in the formation of the acid required to reach the critical pH for enamel decalcification (16). This has been implied from studies using animal models where tooth fissures reacted differently to caries reducing agents and dietary and environmental changes in comparison to smooth tooth surfaces (17).

There is a paucity of information regarding the types, numbers and interrelationships of the organisms involved in the initiation of occlusal decay. A major problem in past investigations of the bacterial colonization of occlusal fissures and subsequent development of dental caries has been the inability of the investigators to obtain samples of the entire fissure.

To resolve this problem, investigators have developed two different approaches using fissure models.

Fejerskov, et al. (18) developed a study model consisting of blocks (2x2x3 mm) made from unerupted third molar occlusal fissures cemented into amalgam restorations of the subject mandibular molars. Theilade, et al. (19,20) in subsequent studies used this technique in trials lasting from one week to 270 days. They reported that after removing the fissure blocks and performing transmission electron microscope studies and microbiological analysis of the fissural contents, definite differences existed in the fissure plaque ecology from that on smooth surfaces.

Löe, et al. (21) turned to fabricating artificial occlusal fissure models from Mylar which were placed in gold inlays and cemented into subjects molars. Theilade, et al. (22) using the Löe model found their samples contained mainly streptococci (i.e., Strep. mutans and Strep. sanguis) with the numbers of lactobacilli increasing over time. In a similar study by Thott, et al. (23) Strep. sanguis and not Strep. mutans was the predominant streptococcus recovered from subjects. Svanberg and Loesche (24), in trying to resolve this apparent discrepancy, related the colonization of artificial fissures to the levels of Strep. mutans found in the subject's saliva. Their report indicated that when the salivary concentrations of Strep. mutans were low initially, the organism did not colonize the fissure.

The results obtained from these fissure model systems while enlightening and interesting, do have their problems and limitations. The use of artificial fissures while providing an improved method of studying the microbiota of this aspect of the tooth, creates an artificial environment for bacterial colonization. The organisms found in the Mylar fissures, over the limited time span of the experiments, may or may not be the ones that actually colonize occlusal fissures and initiate decay in the natural state.

The occlusal fissure block models from human third molars also set up an artificial situation. The fissure block was separated from the rest of the tooth and placed in amalgam. This did not allow the fissures to receive nutrient or fluid from the dentinal tubules which Brown, Wachtel and Wheatcroft (25,26) surmised may be needed to initiate and sustain penetrating decay. Employing this model, no caries developed in the fissure, even rinsing with a sucrose solution twice daily for 270 days (19,20).

This investigation had two major objectives: 1) to develop a method, employing suction, for the collection of the entire contents of occlusal fissures; and 2) to employ this technique for the study of types and numbers of microorganisms implicated in occlusal caries activity (Strep. mutans, Strep. sanguis, Strep. faecalis, lactobacilli and actinomyces).

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Materials and Methods

Subjects - Three groups of male naval recruits, 17-22 years of age were selected for study. The first group consisted of 24 men who had only occlusal lesions present in their mouths. The second group consisted of 24 men who were diagnosed as having both occlusal and proximal decay. An incipient carious fissure from a posterior tooth was removed from each of the latter subjects. Group 1 and 2 were set up to investigate if differences in the percentage of Strep. mutans were present between the fissures taken from patients with only occlusal decay versus patients with both occlusal and proximal decay. The third group consisted of 20 men who had at least one posterior proximal carious tooth surface. A non-carious fissure was removed from each of these subjects when the occlusal extension for access to the proximal lesion was performed. All teeth were previously diagnosed as having either an incipient occlusal lesion or a proximal lesion by an independent examiner. An oral examination and a radiographic review were performed on all subjects prior to fissure removal to verify the suitability of the lesion for inclusion in the study. The Radike (27) criteria for the diagnosis of incipient occlusal caries was used as the standard for selection. There had to be no radiographic evidence of dentinal involvement, and the explorer must have experienced a definite "stick" or catch in the prospective fissure. The final verification of the suitability of the lesion to be classified as "incipient" was made after removal of the carious fissure. A depth, after all caries removal, of no more than approximately 0.5 mm below the dentino-enamel junction (DEJ) was the determinant for final classification of a lesion as incipient. Teeth requiring cavity preparations deeper than 0.5 mm beyond the DEJ or not requiring penetration of the dentin for removal of the fissural decay were excluded for this study. The non-carious fissures were prepared to a depth of approximately 0.5 mm below the DEJ.

Fissure Sampling - The sampling technique consisted of a preparation phase and a sample phase. The preparation phase was concerned with reducing smooth surface plaque and rubber dam bacterial contamination. The sample phase consisted of testing for any residual bacteria surrounding the sample fissure and removing the fissure for collection. Sterile technqieus were observed both during the preparation and sample phases. The operator and assistant were gloved and all instruments had been previously autoclaved (121°C, 15 psi pressure). In the preparation phase the selected tooth was anesthetized using a local anesthetic solution. A rubber dam was placed over the operative quadrant to preclude contamination by the patient's saliva. A prophylaxis to reduce smooth surface plaque was performed on all exposed tooth surfaces using sterile pumice and a bristle brush. Special emphasis was placed on the selected tooth's occlusal surface. The area of the rubber dam surrounding the teeth and all exposed tooth surfaces with the exception of the sample tooth's occlusal surface were swabbed with Lugol's solution for a period of one minute. Sterile water was used to remove the pumice and iodine solution from the sample area. Three microbiologic samples were collected for each fissure removed during the sampling phase. The first sample was taken of the water spray as it emerged from the high-speed handpiece. The second sample was collected as the handpiece was held over the sample tooth and approximately 5 cc's of water spray was allowed to run across the occlusal surface.

These two samples were obtained to detect any bacterial contamination from either the water spray, the surfaces of the sampled tooth, or the surrounding area of the dam. The fissural counts were adjusted by deducting the respective counts from the total or the individual microorganism CFU's as needed. The third sample was taken as the tooth was prepared and the fissure completely removed using a high-speed handpiece with water-air coolant spray, with the fissural contents being collected simultaneously. All preparations were made using a pear-shaped 330* friction grip bur. Samples were collected by suction into a sterile sampling container adapted to fit into the line of a portable suction apparatus. The bur used in removing the tooth's fissure was placed in 2 ml of thioglycollate medium** which acted as a transporting and holding media. The microbial content remaining on the bur would be analyzed employing the same techniques used for the three microbiological samples and its CFU's added to the fissures CFU's for the total and respective organisms recovered.

Culturing of Fissure Samples - The three sample volumes were first measured and then dispersed using a sonifer*** for 5-10 seconds. Prior to dilution and plating, all samples were further dispersed on a vortex mixer for approximately 10 seconds. All samples were serially diluted in 0.05% yeast extract** and plated in duplicate on the following media: blood agar** for total bacterial count; LBS agar** for lactobacilli; PSE agar**** for Strep. faecalis; Mitis-Salivarius agar, with and without bacitracin** for total streptococcal, Strep. sanguis and Strep. mutans; and trypticase soy agar (TSA)** containing 115 ug/ml of sodium fluoride, 20 ug/ml of cadmium chloride (28), 750 ug/ml of crystal violet, and 10 ug/ml of metronidazole (29) for actinomyces. The plates were incubated in a 95 percent nitrogen - 5 percent carbon dioxide atmosphere at 37°C for 48 hours. The LBS and the actinomyces plates were incubated for 72 hours. The number of colony forming units (CFU) were determined for each plate. Gram stains were performed on selected colonies as an aid in identification. The colonial characteristics of Strep. mutans as described by Jordan and his co-workers (30) was used to confirm the presence of this organism. The biochemical scheme described by Shklair and Keene (31) was used in separating the biotypes of Strep. mutans. The biochemical scheme from Bergey's Manual (32) was employed for the differentiation of species of lactobacilli.

Statistical Analysis - Data was compared statistically among the three groups separately and between Groups 1 and 2 (carious fissures collectively) and Group 3 (non-carious fissures). One way analysis of variance was applied to all groups to determine if significant intergroup differences could be detected. If significance was found, nonparametric Kruskal-Wallis and Mann-Whitney U tests were performed. A difference that would occur with $P < 0.05$ was considered significant. Absolute numbers of bacteria were not used in comparing differences between the three test groups because of the great variations in fissure volume (length, width and depth of the enamel-dentin boundaries in which the bacteria were contained) from different teeth. A larger fissure (i.e., molar versus premolar) could possess a greater number of a microorganism because of an increased capacity. Consequently, only the percentage of the total bacterial count of single types or groups of microorganisms were used for intergroup comparisons.

*S. S. White, Philadelphia, PA 19102.
**DIFCO, Detroit, MI 48201.
***Heat Systems - Ultrasonics, Inc., Plainview, N. Y. 10032.
****PFIZER, New York, N. Y. 10017.

Results

Table 1 lists the colony forming units (CFU) that were recovered for all the organisms under investigation. These figures indicate a similar recovery rate with our sampling technique when compared to the rates obtained in the fissure study models of others (18-24). The total counts and the CFU's of specific organisms studied each yielded higher totals in the carious than in the non-carious fissures (Table 1).

Table 2 indicates the percentage of the total count of each organism or group of organisms isolated, as well as the prevalence of individual organisms at the time of sampling. Streptococci accounted for 26.5% of the carious fissural flora, and 19.3% of the non-carious flora. This difference was not statistically significant.

All of the carious fissures had detectable levels of Strep. mutans, whereas three of the 20 non-carious fissures did not harbor these organisms. Strep. mutans comprised 7.3% of the total flora of carious fissures compared to 2.3% of the non-carious flora. This difference was statistically significant. There was a tendency for Group 1 (occlusal caries only) to have more Strep. mutans in its fissures than Group 2 (occlusal and proximal caries), however, this difference was not significant. There was a significant difference in the percentage of Strep. mutans isolated from Group 1, but not Group 2, when compared to Group 3 (non-carious fissures).

Strep. sanguis accounted for a smaller, but statistically non-significant, percentage of the total count in the carious fissures than in the non-carious fissures (Table 2). Strep. sanguis was absent in two of the carious fissures but was present in all the non-carious fissures.

Strep. faecalis was not found in any of the non-carious fissures (Table 2) and only four carious fissures harbored this microorganism. Strep. faecalis comprised the smallest percentage of the total microflora of all the microorganisms under study in the carious fissures. This suggests that Strep. faecalis was not a normal inhabitant of the occlusal fissures and was not a significant contributor to the initiation of occlusal fissures decay among the teeth that were sampled.

When isolated, Actinomyces viscosus comprised a percentage of the total count similar to that for Strep. sanguis in both the carious and non-carious fissures (Table 2). A. viscosus, however, was isolated less frequently than with Strep. sanguis. No significant differences were noted among any of the groups with respect to any of the percentages of A. viscosus.

Lactobacilli were found in 38% of the carious fissures and 15% of the non-carious fissures (Table 2). This was not found to be a statistically significant difference.

The majority of Strep. mutans were identified as biotype c (31) for both the carious and non-carious fissures. The lactobacilli from both the carious and non-carious fissures were identified as predominantly Lactobacillus casei (Table 3).

The contribution of Strep. mutans and Strep. sanguis to the percentage of the total streptococcal count is presented in Table 4. Strep. mutans comprised 24% of all the streptococci in the carious fissures, while accounting for only 11.2% of the streptococci in non-carious fissures. This difference was statistically significant. There was not a statistical difference in the percentages of Strep. mutans between Group 1 and Group 2. There was a trend, however, similar to the one illustrated in Table 2, for a greater number of Strep. mutans to be associated with fissures from mouths experiencing only occlusal decay. Significant differences between the percentages of Strep. mutans were found between Group 1 and Group 3 and between Group 2 and Group 3.

Strep. mutans displayed more than a two-fold increase over Strep. sanguis in carious fissures when their respective contributions to the total streptococcal count were compared (Table 4). Non-carious fissures displayed a reversal of this relationship, where Strep. sanguis comprised over twice the percentage of the total streptococcal population as did Strep. mutans. These differences were statistically significant.

An inverse relationship was noticed when the Strep. mutans to the Strep. sanguis percentages from Tables 2 and 4 were compared. There was a tendency for the Strep. sanguis to decrease as Strep. mutans increased. The Strep. mutans/Strep. sanguis (M/S) ratio, shown in Table 5, depicts this relationship. This ratio was proposed by Loesche (4,33) as a predictor for the cariogenic potential of a fissure. The ratio is derived for each fissure by dividing the percentage of Strep. mutans from the total counts by the corresponding percentage for Strep. sanguis. Using as a reference M/S ratio of one, the greater or lesser the M/S ratio, the greater the difference between the percentages of Strep. mutans to Strep. sanguis in the fissure. The carious fissures had a significant eleven-fold increase in this ratio over the non-carious fissures. No significant difference was noted between the M/S ratios of Group 1 and Group 2. A trend could be seen for higher percentages of Strep. mutans than Strep. sanguis in the fissures of mouths having both proximal and occlusal decay. Both Group 1 and Group 2 demonstrated signi-ficantly different M/S ratios when compared to Group 3.

Table 6 indicates the frequency with which each species of microorganisms comprised the predominant member of the fissural microflora, at the time of sampling. In the carious fissures Strep. mutans was the predominant organism in the highest percentage of fissures when A. viscosus held this position in the non-carious fissures.

Discussion

The data from this investigation presents a strong case for the role that Strep. mutans plays in the initiation of incipient occlusal fissure decay. It does not, however, rule out the role of other microorganisms, either by themselves or in conjunction with Strep. mutans, in initiating fissure decay.

If one assumes that in a carious fissure the organism in greatest numbers is the most likely candidate to be the prime odontopathogen, then Strep. mutans would be that candidate. Strep. mutans comprised the majority of the bacterial population in 48% of the carious fissures compared to 25% of the

non-carious fissures (Table 6). Strep. mutans and lactobacilli were the only microorganisms studied which displayed an increase in the frequency of comprising the predominant member of the fissural population when comparing non-carious and carious fissures. Strep. sanguis and A. viscosus decreased in the frequency in which they comprised the majority of the bacterial population when comparing non-carious and carious fissures.

Strep. mutans was always associated with occlusal decay, and was the only organism studied that displayed this relationship. However, Strep. mutans was also present in 85% of the non-carious fissures; in 25% of the cases Strep. mutans was present in greater total numbers than in the carious fissures.

Considering the results obtained in this study, one cannot state with certainty that Strep. mutans is the sole member of the fissural flora responsible for initiating fissural decay. Strict anaerobic techniques might divulge different species that are important. The wide variations in the amounts of microorganisms found in both carious and non-carious fissures (represented in the tables by the large standard deviations) are similar to those observed in previous fissure studies (18-23). This may indicate that each fissure is a unique ecosystem in itself and many factors play a role in determining whether a fissure will or will not become carious. This is further demonstrated by examining the Strep. mutans/Strep. sanguis (M/S) ratios of the carious and non-carious fissures (Table 5). Loesche's (4,33) assumption that a ratio greater than one is indicative of a strong potential for caries to develop, appears generally accurate. However, this does not hold true for all carious and non-carious fissures when examined individually. Thirty-eight percent of the carious fissures displayed an M/S ratio less than one. The M/S ratios of 25% of the non-carious fissures and the overall average ratio for the group was greater than one. Whether other modifying factors in the fissure prevented decay in the presence of increased amounts of Strep. mutans in relation to Strep. sanguis is not known. Perhaps these fissures, in time, will become carious.

No clear statistical differences were noted in the quantity of Strep. mutans found in the fissures of patients with only occlusal decay present, versus those with both occlusal and proximal lesions. However, a trend was present which indicated that greater quantities of Strep. mutans were associated with those fissures in patients with only occlusal decay. This is contrary to what one would suspect because of the strong association of Strep. mutans in smooth surface decay (4-6). There should have been more Strep. mutans in the mouths of the patients with proximal decay than just occlusal decay and hence a greater chance for increased seeding of Strep. mutans into these patient's fissures. The reason for this unexpected result is not known.

The present investigation provided for the first time the removal for study of the entire incipient carious fissure. This data in conjunction with previous fissure investigations of Loesche (4,33) and Ikeda, Sandham, and Bradley (7) increase the understanding of the microorganisms implicated in the carious process in the most caries-prone areas of the tooth.

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The complexity of fissural decay and its impact on time and resources of naval personnel necessitates that further investigations are needed to enhance the understanding of the etiology of fissural decay specifically and tooth decay in general. Clinical trials utilizing therapeutic agents which can effectively eliminate all viable microflora in incipient fissural decay should be undertaken. Use of these agents would prove less time consuming and more cost-effective than the currently employed restorative procedures used to eliminate carious fissures. If an effective, inexpensive method of prevention or treatment could be discovered, a significant reduction in man-days lost for restorative treatment and an enhancement of the combat readiness of Navy and Marine Corps personnel would be realized.

Summary

Pits and fissures of teeth represent the most common sites for dental decay, yet these are areas where preventive measures are least effective. These sites are also difficult to sample, consequently, there is a paucity of information on bacterial colonization and initiation of the decay process.

This investigation had two major objectives: 1) to develop a method for the collection of the entire bacterial contents of occlusal fissures, and 2) to use this technique for the study of the type and numbers of microorganisms found in incipient occlusal fissure decay.

The fissure sampling technique consisted of: 1) isolation of the sample teeth with a rubber dam, 2) removal of all surface contamination from the teeth and surrounding rubber dam, and 3) removal of the fissure by high-speed hand-piece and water spray with suctioning of the fissural contents into a sterile sampling container.

A total of 68 teeth from naval recruit volunteers were sampled, using this technique. Forty-eight of these teeth had occlusal fissure decay and 20 had no occlusal fissure decay.

The total number of microorganisms removed from the decayed fissures was four times the total number recovered from the non-decayed fissures. Strep. mutans was the only microorganism under investigation which was recovered from all decayed fissures, found in significantly ($p < 0.05$) greater numbers in decayed than in non-decayed fissures, and was, most often, the predominant member of the bacterial population in decayed fissures.

From this data, Strep. mutans appears to play an important role in the initiation of decay in occlusal fissures. This data, however, does not solely implicate Strep. mutans in occlusal decay, nor rule out the possibility of other organisms contributing to, or being an important factor in the decay process of occlusal fissures.

The complex nature of occlusal decay warrants further study to increase the understanding of its cause so that more efficient means of prevention and treatment can be developed. An effective, inexpensive method of treating or preventing incipient fissure decay would result in a significant reduction in man-days lost for restorative treatment and enhance the combat readiness of Navy and Marine Corps personnel.

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TABLE 1

Microorganisms Isolated from Occlusal Fissure Samples

	CARIOUS			NON-CARIOUS
	Group 1* (N=24) CFU	Group 2* (N=24) CFU	Total* (N=48) CFU	Group 3* (N=20) CFU
Total count	435.0 ± 891.0	214.0 ± 263.0	320.0 ± 660.0	80.3 ± 127.0
Total streptococci	35.6 ± 56.3	56.6 ± 84.9	46.1 ± 72.0	12.3 ± 21.0
S. mutans	13.8 ± 32.0	9.3 ± 17.1	11.5 ± 25.5	1.8 ± 2.1
S. sanguis	2.7 ± 4.5	2.7 ± 4.2	2.7 ± 4.3	2.3 ± 5.0
S. faecalis	0.0001 ± 0.0007	0.02 ± 0.07	0.01 ± 0.05	0
A. viscosus	8.3 ± 35.6	6.1 ± 13.4	7.2 ± 26.6	2.8 ± 6.5
Lactobacilli	4.7 ± 18.8	5.9 ± 18.4	5.3 ± 18.6	0.005 ± 0.017

*Values shown are the mean ± S.D. ($\times 10^4$)

Group 1 - Occlusal caries only
Group 2 - Occlusal and proximal caries

TABLE 2

The Mean Percent of Microorganisms Isolated From Occlusal Fissure Samples

	CARIOUS						NON-CARIOUS	
	Group 1 (N=24)		Group 2 (N=24)		Total (N=48)		Group 3 (N=20)	
	%	No. Pos. Cultures	%	No. Pos. Cultures	%	No. Pos. Cultures	%	No. Pos. Cultures
Total streptococci	26.1 ± 19.6	24	26.8 ± 17.9	24	26.5 ± 18.6	48	19.3 ± 4.2	20
mutans	$8.9 \pm 11.0*$	24	5.8 ± 7.7	24	$7.3 \pm 9.5†$	48	$2.3 \pm 2.9*†$	17
sanguis	2.9 ± 2.6	23	2.2 ± 3.0	23	2.1 ± 2.8	46	3.4 ± 3.7	20
mitis	0.00001 ± 0.00004	1	0.01 ± 0.02	3	$0.004 \pm .02$	4	0	0
viscosus	1.6 ± 2.4	18	3.2 ± 5.2	22	2.4 ± 4.1	40	3.3 ± 5.6	15
Lactobacilli	0.4 ± 1.0	9	1.4 ± 4.7	9	0.9 ± 3.4	18	0.02 ± 0.06	3

* Significant Mann-Whitney U, $P<0.05$

† Significant Mann-Whitney U, $P<0.0?$

Group 1 - Occlusal Caries Only

Group 2 - Occlusal & Proximal Caries

TABLE 3

Biotypes of Streptococcus mutans and Species of Lactobacilli
from Occlusal Fissure Samples

	CARIOUS FISSURES (N=48) No. of Isolates	NON-CARIOUS FISSURES (N=20) No. of Isolates
Biotypes of S. mutans	47 c, 2 e	14 c, 2 d, 1 e
Species of Lactobacilli	14 L. casei 2 L. plantarum 1 L. brevis 1 L. acidophilus	3 L. casei

TABLE 4

Streptococcus <u>mutans</u>, Streptococcus <u>sanguis</u>, Streptococcus <u>faecalis</u>: Mean Percentage of the Total Streptococcal Count

| | CARIOUS | | | NON-CARIOUS |
	Group 1 (N=24) %	Group 2 (N=24) %	Total (N=48) %	Group 3 (N=20) %
S. mutans	28.3 ± 26.7†	20.1 ± 17.9*	24.2 ± 22.9Δ	11.2 ± 14.0†*Δ
S. sanguis	10.3 ± 10.9†	9.7 ± 11.2**	9.9 ± 10.9ΔΔ	24.3 ± 25.9††**ΔΔ
S. faecalis	0.001 ± 0.01	0.07 ± 0.21	0.03 ± 0.15	0

† Significant difference Mann-Whitney U, P<0.01

* Significant difference Mann-Whitney U, P<0.05

†† Significant difference Mann-Whitney U, P<0.01

** Significant difference Mann-Whitney U, P<0.01

Δ Significant difference Mann-Whitney U, P<0.05

ΔΔ Significant difference Mann-Whitney U, P<0.05

TABLE 5

S. mutans/S. sanguis Ratios

	CARIOUS						NON-CARIOUS	
	Group 1 (N=24)		Group 2 (N=24)		Total (N=48)		Group 3 (N=20)	
	x̄ + S.D.	No. of M/S Ratios>1	x̄ + S.D.	No. of M/S Ratios>1	x̄ + S.D.	No. of M/S Ratios>1	x̄ + S.D.	No. of M/S Ratios>1
	9.3 + 11.7*	16	18.8 + 30.6+	14	14.0 + 23.4**	30	1.5 + 2.9*+**	5

* Significant difference Mann-Whitney U, P<0.01   Group 1 - occlusal caries only

+ Significant difference Mann-Whitney U, P<0.005   Group 2 - occlusal and proximal caries

** Significant difference Mann-Whitney U, P<0.001

TABLE 6

The Frequency with which the Organism was the Predominant Member of the Fissure Flora

| | CARIOUS | | | NON-CARIOUS |
	Group 1* (N=24) %	Group 2** (N=24) %	Total (N=48) %	Group 3 (N=20) %
S. mutans	54	42	48	25
S. sanguis	21	8	15	30
S. faecalis	0	0	0	0
A. viscosus	17	29	22	40
lactobacilli	8	21	15	5

*Group 1 - occlusal caries only
+ Group 2 - occlusal and proximal caries

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The bacterial contents of occlusal surface pit and fissure incipient dental caries was studied, following excavation of the fissures in naval recruits. Streptococcus mutans was recovered from all carious fissures and in significantly greater numbers in the carious fissures than from non-carious fissures, and was most often the predominant member of the bacterial population in carious fissures.

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